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Comparative Tissue Distribution of Mirex and Chlordecone in Fetal and Neonatal Rats

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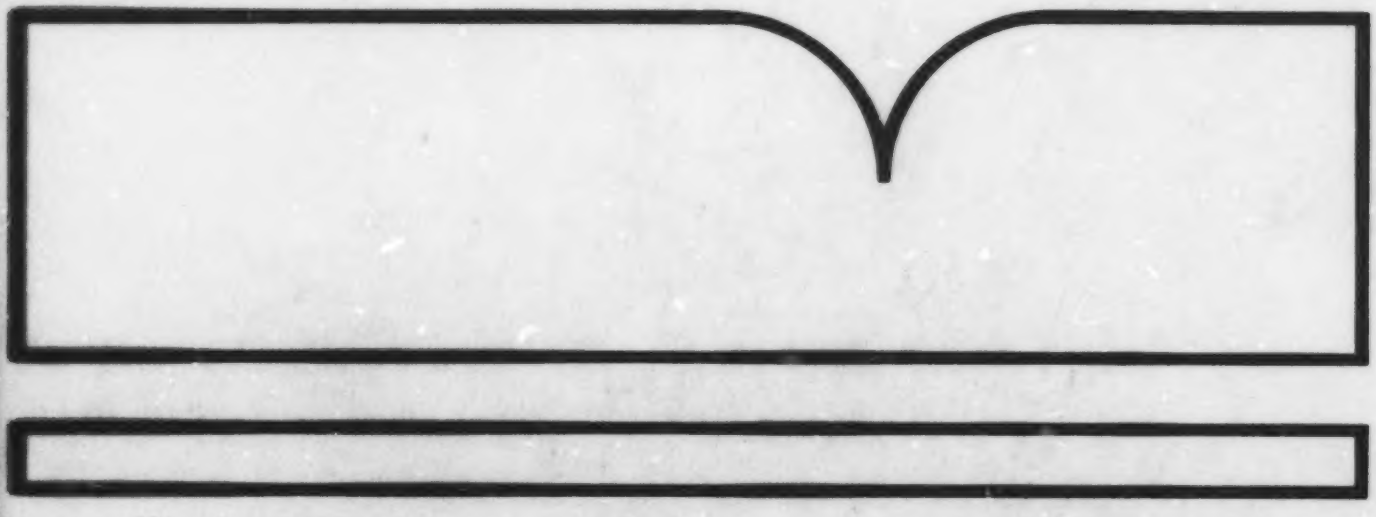

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16. ABSTRACT

The transport of mirex and chlordecone (Kepone) across the placenta during late gestation and through the milk during lactation was investigated in the rat. In the placental transport study, doses of 5 mg/kg were administered on Day 15, 18 or 20 of gestation and animals were killed 4, 24, or 48 hr after treatment. Both compounds crossed the placenta and were present in the fetus at all examination times. Maternal tissue levels exceeded fetal tissues. No effects of gestational age at time of treatment or of the fetus in the uterus were seen. In the lactation study, doses of 1 or 10 mg/kg/day were administered on Days 2-5 postpartum and pups were killed at intervals up to 12 days after treatment. The secretion of milk appeared to be a major route of elimination for both pesticides for nursing females, and the greater amount of mirex excreted via the milk as compared with chlordecone is in agreement with differences in their reported octanol-water partition coefficients. Initially, mirex entered the milk more rapidly than chlordecone. After cessation of treatment, mirex milk levels fell quickly, but chlordecone levels remained fairly constant. In the pups, mirex tissue levels paralleled milk levels; chlordecone levels, however, continued to increase in the tissues throughout the observation period.

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Journal Article

Comparative Tissue Distribution of Mirex and Chlordecone in Fetal and Neonatal Rats

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The transport of mirex and chlordecone (Kepone) across the placenta during late gestation and through the milk during lactation was investigated in the rat. In the placental transport study, doses of 5 mg/kg were administered on Day 15, 18, or 20 of gestation and animals were killed 4, 24, or 48 hr after treatment. Both compounds crossed the placenta and were present in the fetus at all examination times. Maternal tissue levels exceeded fetal tissue levels by a factor of 4 to 5. Slightly higher levels of chlordecone as compared to mirex were found in maternal and fetal tissues. No effects of gestational age at time of treatment or of position of the fetus in the uterus were seen. In the lactation study, doses of 1 or 10 mg/kg/day were administered on Days 2–5 postpartum and pups were killed at intervals up to 12 days after treatment. The secretion of milk appeared to be a major route of elimination for both pesticides for nursing females, and the greater amount of mirex excreted via the milk as compared with chlordecone is in agreement with differences in their reported octanol–water partition coefficients. Initially, mirex entered the milk more rapidly than chlordecone. After cessation of treatment, mirex milk levels fell quickly, but chlordecone levels remained fairly constant. In the pups, mirex tissue levels paralleled milk levels; chlordecone levels, however, continued to increase in the tissues throughout the observation period.

INTRODUCTION

Mirex (decachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentane) and chlordecone (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one, more commonly known by its tradename, Kepone) are two structurally similar insecticides, differing only in the replacement of two chlorine atoms in mirex by a more polar carbonyl group in chlordecone. This structural difference leads to marked dissimilarities in their biological activities. Chlordecone, but not mirex, is uterotrophic in weanling rats and induces precocious vaginal opening, persistent vaginal estrus, and anovulation (1). Mirex, but not chlordecone, impairs the biliary excretion of sulfobromophthalein in rats (2, 3). Exposure to chlordecone results in central nervous system toxicity including tremors, effects which are absent in mirex-treated animals (4). Administration of mirex to pregnant rats readily induces a pronounced edema in the fetuses (5) while chlordecone does so only to a lesser extent (6). Lactational exposure to mirex, but not chlordecone, induces the formation of cataracts in rats and mice (7, 8). Other aspects of the toxicology of these two compounds can be found in recent reports and reviews (9–11).

Since neither mirex nor chlordecone undergoes metabolism in mammalian systems (12–16), the analysis of tissue levels based upon the detection of ^{14}C-labeled compounds without the confounding effects of metabolic products is possible. We took advantage of this property in examining the behavior of these compounds in the maternal/fetal and maternal/pup units.

METHODS

Time pregnant CD rats, 80–90 days of age, were obtained from Charles River Breeding Laboratory, Wilmington, Massachusetts. Day 1 of pregnancy was recorded upon demonstration of sperm in a

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vaginal smear. Animals were housed individually in polypropylene cages on pine-shaving bedding. Light (12 hr), temperature (22–24°C), and humidity (60%) were kept constant. Animals were allowed free access to food and water. Uniformly labeled [^{14}C]-chlordecone and [^{14}C]mirex with specific activities of 3.8 and 0.9 μCi/mg, respectively, were used.

Prenatal studies. Pregnant rats were dosed on Days 15, 18, or 20 of gestation with 5 mg/kg of either chlordecone or mirex. These days of administration were chosen in order to spread exposure over as much of gestation as possible, while at the same time allowing examination of fetal organ burdens. The dose was based on the animal's weight immediately prior to oral intubation. Corn oil was used as the solvent, and the intubation volume was 0.2 ml/100 g body wt. Animals were killed by an overdose of pentobarbital at 4, 24, or 48 hr after dosage with the exception of the groups treated on Day 20 where parturition prevented the 48-hr sacrifice. Samples (approximately 100 mg) of maternal liver and kidney were weighed and immediately frozen. Maternal blood was collected by internal cardiac puncture in a heparinized syringe. Plasma was separated by centrifugation at 5000*g* for 10 min. A 50-μl sample was then taken and analyzed. The fetal tissues (whole brain above medulla, heart, liver, kidney, and dorsal fat pad) were collected from three fetuses of each pregnant rat sacrificed. The fetuses chosen comprised the most anterior, a medial, and the most posterior in the left uterine horn. In younger fetuses some of these organs could not be obtained and in those instances the fetal head (above the jaw), liver, heart, and trunk (the remainder of the carcass) were utilized. The tissues were weighed and frozen. Radioactivity was determined by liquid scintillation counting after oxidation of the tissues (Packard Tri-Carb Oxidizer, Model B306). Concentrations of chlordecone and mirex were then expressed as parts per million based on the wet weight of the tissues. The effects of gestational day at time of administration of the pesticides and the hour of observation on the amount of substance present in maternal and fetal tissues were analyzed by a two-way analysis of variance.

Postnatal studies. Pregnant rats were allowed to give birth, at which time the litters were randomly reduced to eight pups. Day 1 of lactation was considered to be the day when all litters had completed parturition. The dams were dosed with either 1 or 10 mg/kg/day of chlordecone or mirex on Days 2 to 5. Maternal weight on Day 2 was used as the basis for the calculation of dosages. The pesticides were administered as described above. On Days 3, 5, 7, 9, 12, 15, and 17 of lactation, single pups from each of five litters in each treatment group were removed and killed by decapitation. Samples of brain (cerebral hemisphere), kidney (posterior cortex), liver (posterior lobe), fat (epididymal), eye (the entire capsule carefully trimmed of fat), and stomach milk were weighed, frozen, oxidized, and radioactivity was determined as described above. In addition, on Days 5, 9, and 15 of lactation, the dams were given an injection of oxytocin and milk was sampled directly from the teat. Concentrations of the pesticides in maternal milk were then compared with those recorded for milk sampled from the stomachs of pups.

RESULTS

Prenatal Administration

Maternal. The distribution of mirex and chlordecone in the maternal animal is summarized in Fig. 1. Mirex, and to a lesser extent chlordecone, readily entered the maternal blood stream. Four hours after oral intubation on Day 15 of gestation plasma concentrations were 13 and 6 ppm for mirex and chlordecone, respectively. There were no significant effects of day of administration or hour of observation on plasma or tissue concentrations of chlordecone. The renal to plasma tissue concentration ratio averaged 0.96, while the he-

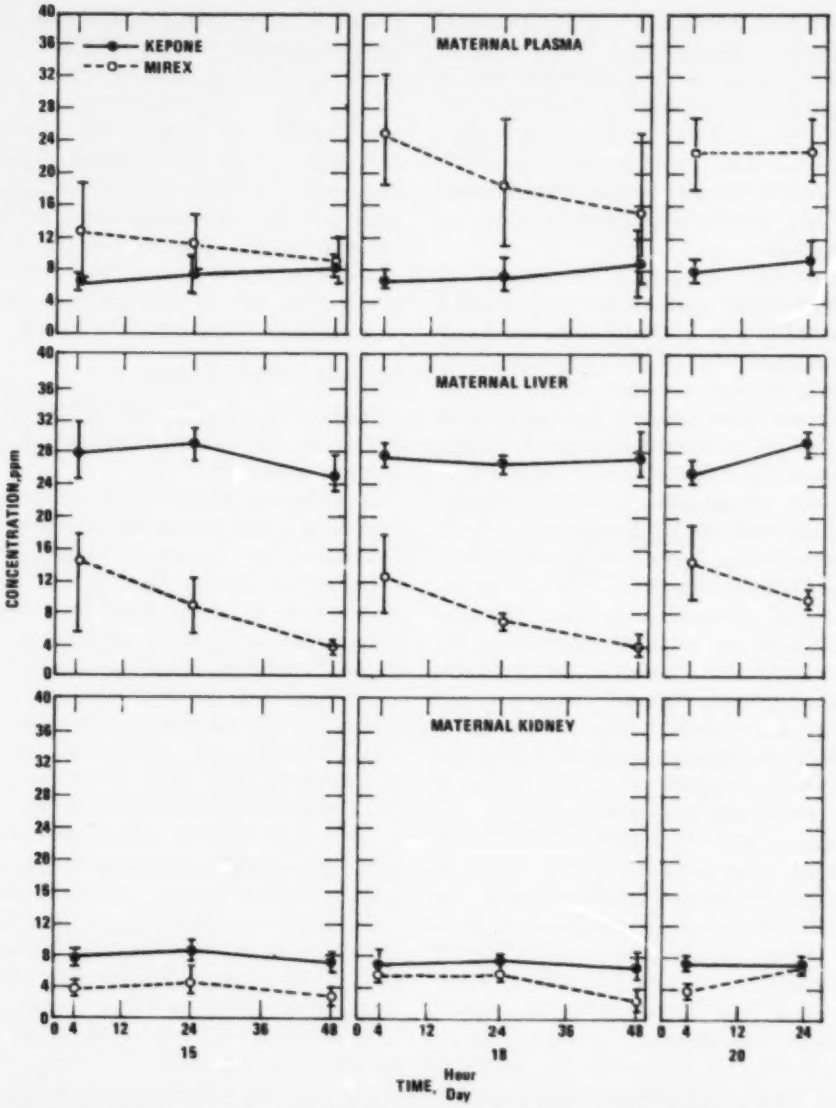

FIG. 1. *Concentrations of mirex and chlordecone in maternal tissues examined 4, 24, or 48 hr after receiving a dose of radioactive substance on either Day 15, 18, or 20 of gestation. Data are from four animals at each point. Vertical bars represent 1 SD unit.*

patic to plasma tissue concentration ratio averaged 3.6. Unlike chlordecone, mirex plasma concentrations were significantly effected by both the time of administration and hour of observation ($P < 0.01$). Plasma concentrations were higher with administration at older gestational ages (13 ppm 4 hr after administration on Day 15 compared to 23 ppm on Day 20) and declined with time after dosing. Concentrations of mirex in the liver also declined with time after administration ($P < 0.01$), but there was no

effect of gestational age at time of treatment on hepatic concentrations. Renal concentrations of mirex were similar regardless of the age at administration or hour of observation. While plasma levels for mirex were often more than double that for chlordecone, hepatic and renal concentrations were only about one-half that recorded for chlordecone.

Fetal. The distribution of chlordecone and mirex in the placenta and fetal organs is presented in Fig. 2. In the placenta, maximal concentrations of both pesticides were between 3.5 and 4.0 ppm. These concentrations are approximately one-half of those recorded in the maternal plasma for chlordecone and one-third those for mirex. Concentration of chlordecone in the placenta remained fairly steady for up to 48 hr after treatment, while mirex levels at 48 hr after dosing were less than 50% of the 4-hr level.

Chlordecone levels in the fetus were generally highest in the liver followed by the brain, heart, and kidney. Concentrations rose during the first 24 hr after dosing and declined in the second 24-hr period regardless of gestational age at time of dosing. Because the fetal organs are in a rapid-growth phase during late gestation, significant new deposition of chlordecone was occurring for organ concentrations remain steady or actually rise with time after treatment. For example, total hepatic chlordecone levels were 193 ng 4 hr after treatment on Day 18, 478 ng after 24 hr, and 586 ng after 48 hr. Samples of fetal dorsal fat pad averaged 3 ppm 4 hr after treatment on Day 20 and 8 ppm 24 hr after treatment. No effect of uterine position on chlordecone concentrations in fetal organs was evident.

Fetal organ concentrations of mirex showed a temporal sequence similar to that noted for chlordecone. Organ concentrations were relatively low 4 hr after dosing, rose slightly at 24 hr, and declined thereafter. The decline noted in the second 24-hr period was due to both organ growth and mirex elimination, as total organ burdens declined during this period. The relative uptake of mirex by the fetal organs was in the order of liver > brain = heart > kidney in an approximate ratio of 3:2:2:1. Mirex levels in the fetal fat pad were less than 0.1 ppm 4 hr after treatment on Day 20 and rose to 4 ppm after 24 hr. Maximal concentrations for all organs were lower than recorded in the chlordecone experiment. As was the case for chlordecone, the position of the fetus in the uterine horn did not appear to affect the amounts or concentration of mirex recorded in the fetal tissues.

Postnatal Administration

Data on the organ uptake of chlordecone and mirex subsequent to lactational exposure are presented in Fig. 3.

Chlordecone. Chlordecone administered to nursing dams entered the milk in proportion to the dose. Females in the high-dosage group (10 mg/kg/day) had milk levels approximately 10 times higher than those in the low-dosage group (1 mg/kg/day). Peak stomach milk concentrations for both dosage groups were obtained on Day 7 of lactation (2 days after cessation of dosing). There was a steady elimination of chlordecone via the milk. Concentrations on Day 15 of lactation were reduced by only a factor of ½ over maximal concentrations. The prolonged appearance of chlordecone in the milk resulted in steady increases in neonatal organ concentrations despite the rapid growth of those organs. Organ burdens, which were still increasing on the last day of observation, rose from 2 μg on Day 5 to 23 μg for the liver and from 16 to 150 μg for the brain in pups from the high-dosage group. The concentration of chlordecone in the eyes of animals in that group ranged from undetectable on Day 3 to 5 ppm on Day 9; levels of 74 ppm were recorded in the fat on Day 17. Uptake was in the order of liver > brain > eye by factors of approximately 16:4:1.

Mirex. As with chlordecone, mirex appeared in the milk in direct proportion to the dosage. Mirex, however, entered the milk supply at a faster rate than recorded

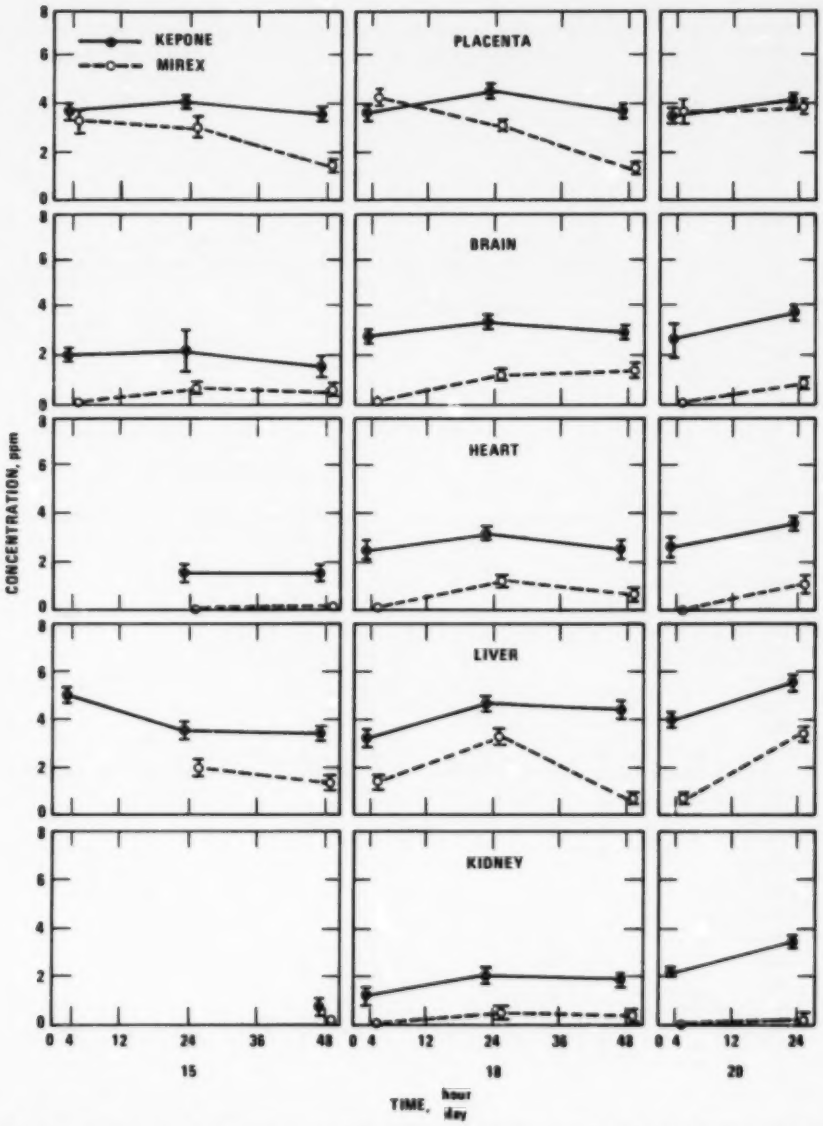

FIG. 2. *Concentrations of mirex and chlordecone in fetal tissues examined 4, 24, or 48 hr after the dam had received a dose of radioactive substance on either Day 15, 18, or 20 of gestation. Data are from 12 fetuses at each point. Vertical bars represent 1 SD unit.*

for chlordecone. Pup stomach milk contents recorded on Day 5 of lactation were six times higher than similarly dosed chlordecone animals. Unlike chlordecone, mirex levels in the milk rapidly declined after cessation of dosing. On Day 7 (when chlordecone milk levels were at their peak), mirex milk levels were only one-half the Day 3 level. Concentrations of mirex in the liver reflected changing milk concentra-

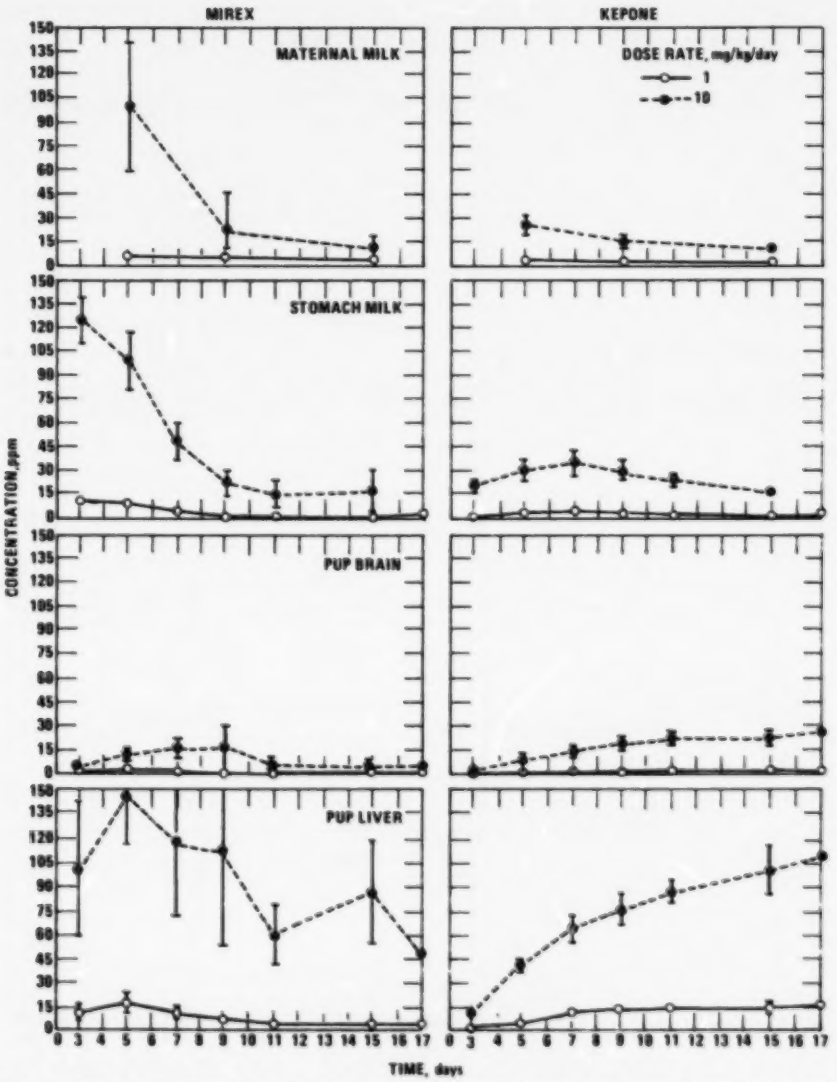

FIG. 3. *Concentrations of mirex and chlordecone in maternal milk, pup stomach milk, brain, and liver following administration to the lactating females on Days 2–5 of lactation. Data are from five nonsibling pups at each point. Vertical bars represent 1 SD unit.*

tions. The peak liver concentration was reached on Day 5 and had declined to less than one-half by Day 11. Concentrations rose more slowly in the brain and eyes, reaching a peak around Day 7. The peak concentration in the eye was 3 ppm, and the levels in the eyes never differed appreciably from those recorded for chlordecone. On Day 7, the approximate ratio of mirex in the liver, brain, and eyes was 40:4:1. The peak hepatic organ burden was reached on Day 7, with amounts of 80 and 8 μg recorded for the high- and low-dosage groups, respectively. These burdens did not change ap-

preciably throughout the remainder of the experiment. The organ burden for the brain reached a peak of 5 μg for the high-dosage group on Day 9 and then gradually declined to 3 μg by Day 17.

DISCUSSION

As with other aspects of their biological activities, the tissue distributions of mirex and chlordecone presented some marked differences. In the gravid female, chlordecone (the more polar compound) entered the maternal plasma to a lesser extent than did mirex, and chlordecone tissue concentrations remained fairly constant throughout the 48-hr-observation period whereas mirex tissue concentrations (with the exception of fat) declined considerably. The slower disappearance of chlordecone versus mirex from the tissues is supported by the reported first half-lives in the rat of 8.5 days (17) and 38 hr (14), respectively. In contrast to mirex, where hepatic levels were either less than or equal to plasma levels, there was a greater than 3:1 ratio of hepatic to plasma chlordecone concentration gradient. Although the placenta posed a barrier to the passage of both compounds (fetal hepatic concentrations were roughly one-third and one-fifth that observed in the maternal livers for mirex and chlordecone, respectively) significant amounts of mirex and chlordecone were present in fetal tissues within 4 hr of oral dosing. Similar to the pattern observed for maternal tissues, chlordecone concentrations in the fetal tissues remained fairly constant during the period of observation indicating that significant deposition had occurred since this was a time of rapid organ growth. In contrast, mirex concentrations tended to peak by 24 hr after dosage and decline thereafter. There were no apparent differences in concentration of either compound in tissues from fetuses located at distinctly different regions of the uterus (anterior, medial, or posterior) or in the tissues from different age fetuses when examined at equal times after dosing.

Khera *et al.* (5) reported on concentrations of mirex in various maternal and fetal tissue in the rat on Day 22 of gestation after treatment with between 1.5 and 12.5 mg/kg/day on Days 6–15. Tissue concentrations were understandably lower than in the present study (due to the length of time between termination of exposure and collection of tissues), but the gradient between the maternal and fetal tissues were similar (e.g., approximately 2:1 for the liver in the Khera study as compared with approximately 5:1 in the present study).

As is the case with many chlorinated hydrocarbons, fecal excretion is the primary route of elimination in adult animals for both chlordecone (17) and mirex (14). It appears, however, that for the nursing females milk secretion may play an important role in excretion of these pesticides. Using the estimates of Bornschein *et al.* (18) for daily consumption of milk by neonatal rats, the data in the present experiment for concentration of the pesticides in the milk and litter size, and assuming that values for milk consumption and pesticide content can be linearly extrapolated between measurement days, it can be calculated that females in our experiment excreted a total of approximately 11,000 μg of mirex and 6000 μg of chlordecone via the milk during the entire lactation period. This represents 95 and 52% of the total amount of mirex and kepone administered, respectively. While these figures are rough estimates based on several assumptions and could be subject to considerable error, they indicate that large amounts of both pesticides are excreted via the milk. These estimates compare with cumulative excretion of chlordecone via the feces of 33% two weeks after administration (17) and 58% for mirex after 7 days (14). The greater total excretion of mirex via the milk as compared to chlordecone is in agreement with the greater octanol–water partition coefficient of mirex versus chlordecone (logarithms of the partition coefficients for mirex and chlordecone are 6.89 and 5–5.5, respec-

tively (19; G. D. Veith, personal communication).

The comparative neonatal tissue distributions of mirex and chlordecone differed greatly in neonates receiving lactational exposure to these compounds. Both compounds entered the milk supply, but the entry of mirex proceeded at a much higher rate. Stomach milk contents in animals from the 10 mg/kg/day mirex group contained 99 ppm on Day 5 as compared with only 30 ppm in animals from the 10 mg/kg/day chlordecone group. Mirex concentrations in the milk declined much more rapidly than did chlordecone milk concentrations. By Day 11, mirex concentrations from stomach milk in the 10 mg/kg/day group had fallen to less than 16% of the Day 5 levels, whereas chlordecone milk concentrations from animals in the 10 mg/kg/day group were reduced to only 78% of the Day 5 levels. Such differences in the appearance of chlordecone and mirex in the milk led to striking differences in the concentration of these compounds in neonatal tissues. Tissue concentrations of chlordecone, due to prolonged excretion via the maternal milk, continued to rise long after administration to the mothers ceased. On Day 11, the concentration of chlordecone in the liver was greater than twice as much and the total hepatic burden was more than four times as much as observed on Day 5. Mirex tissue concentrations, on the other hand, peaked soon after cessation of maternal dosing, and had declined considerably by Day 11. It appears that mirex, the less polar of the two compounds, was able to penetrate into the milk more readily than chlordecone, and by this and perhaps other routes was more rapidly eliminated from the body. These differences would be reflected in the prolonged appearance of chlordecone in the milk as compared to mirex and in the pattern of tissue concentrations observed in pups consuming that milk. Of final note are the observations that pup stomach milk contents appear to adequately reflect maternal milk levels (at least for these two compounds), thus eliminating the need for the dual samplings and that there are no differences in the contents of the two compounds in the eye, indicating that the lack of cataractogenicity of chlordecone versus mirex is not due to its relative inability to penetrate into that tissue.

ACKNOWLEDGMENTS

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